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14. ABSTRACT

Toll-like receptors (TLRs) are key signaling molecules that regulate innate and adaptive immune responses to the presence of pathogens. The role of TLRs in cancer is unclear. During our first year of this training proposal, we are expanding transgenic and transgenic crosses to various TLR signaling molecules for Specific Aims 1 and 2. We have generated TRAMP Tg+/- x MyD88-/- mice. Initial results reveal that de novo prostate cancers in absence of MyD88 are larger with higher grade adenocarcinomas than wild-type controls. Analysis of tumor infiltrating cells reveals increased infiltrating macrophage lineage in the absence of MyD88. We are in the process of understanding the activation of signaling pathways, local and systemic cytokine levels, and other infiltrating cell types that may modulate the differences in tumor development. We are also isolating TRAMP Tg/- cell lines deficient in MyD88 to ascertain the role of MyD88 in the intrinsic growth of TRAMP prostate cancer cells.

15. SUBJECT TERMS
Prostate cancer, tumor microenvironment, Toll-like receptors

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Introduction

Inflammation has long been associated with the prostate cancer microenvironment. However, the contribution of the tumor infiltrating lymphocytes (TILs) to prostate cancer development, growth, and metastasis is unclear. We are interested in understanding the mechanisms for development of TILs and how they modulate prostate cancer. Our hypothesis is that TILs play a key role in tumor surveillance, are important in generation of tumor-specific immunity, and that by modulating the composition of TILs, we can alter tumor growth.

Pathogens or cancerous cells alike can produce danger signals that elicit the activation of immune responses. These signals in the form of conserved molecules termed pathogen-associated molecular patterns (PAMPs) or danger-associated molecular patterns (DAMPs) can be discriminated from self-antigens by a family of pattern-recognition receptors, including Toll-like receptors (TLRs). Thirteen mammalian TLRs have been identified to date with ligands ranging from lipopolysaccharide (LPS) found in gram-negative bacterial walls recognized by TLR4, double stranded RNA produced by many viruses for TLR3, viral CpG motifs with TLR9, to endogenous ligands such as heat-shock protein 70 and chromatin component HMG-B1. Activation of these receptors leads to induction of multiple inflammatory pathways, including nuclear factor-kappa B (NF-κB) and interferon regulatory factors (IRFs), mediating the development of cytotoxic T lymphocytes (CTLs) and dendritic cell (DC) maturation[1]. Although TLRs have been shown to inhibit negative regulatory cells such as Tregs, the relationship between TLRs and myeloid-derived suppressor cells (MDSCs) is less clear[2, 3].

TLRs recruit adaptor proteins such as MyD88 and serine kinase IL-1 receptor-associated kinase (IRAK), leading to activation of MAP kinases, NF-κB, and expression of inflammatory

genes. Most TLRs utilize the MyD88 pathway. The role of TLRs in modulating cancer is conflicting, as prior reports have suggested tumor promoting as well as suppressing effects. Deficiency in MyD88 confers decreased development of tumors in a mouse model of spontaneous intestinal tumorigenesis and diethylnitrosamine-induced hepatocellular tumors.

Body

Currently, we are expanding our TRAMP x FvB colony as well as breeding TRAMP Tg+/- x MyD88-/- and TRAMP Tg+/- x TRIF-/- mice. We have successfully bred TRAMP Tg+/- x MyD88-/- mice and when compared to TRAMP Tg+/- animals, have larger de novo prostate tumors and more aggressive histology (Figure 1). We are generating TRAMP Tg x MyD88-/- cell lines to determine the intrinsic role of Myd88 in contributing to this difference in prostate cancer growth and development. This will be compared with the role of MyD88 in the microenvironment and systemically. We have also examined expression of the T lymphocyte antigen CD3, macrophage antigen F4/80, and the proliferative marker Ki67 in 24 week wild-type and MyD88 prostate tumors to examine the microenvironment (Figure 2). We show decreased infiltration of CD3 lymphocytes with concomitant increase in infiltrating F4/80 cells. This could suggest the infiltration of myeloid derived suppressor cells that can negatively regulate T cells and are currently being investigated.

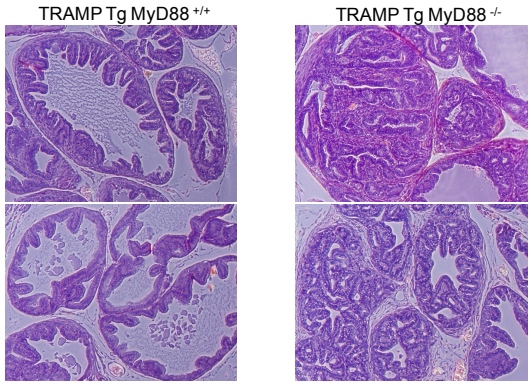

Figure 1. Larger and more aggressive adenocarcinoma in 24 wk-old C57Bl6 TRAMP $Tg^{+/-}$ transgenic mice compared to age matched C57Bl6 TRAMP $Tg^{+/-}$ x $MyD88^{-/-}$ mice with H&E staining at 20x magnification.

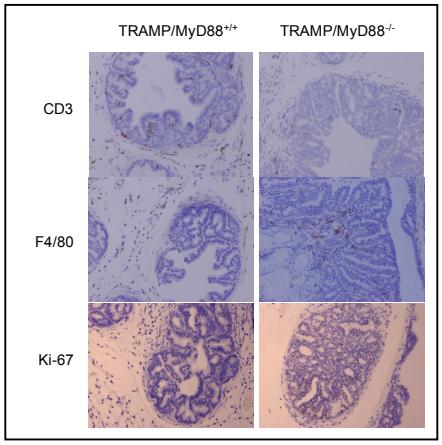

Figure 2. Inflammatory infiltrate in autochthonous prostate cancer in absence of MyD88. Infiltration of CD3, F4/80, and Ki-67 examined by immunohistochemistry in 24 wk-old C57Bl6 TRAMP $Tg^{+/-}$ transgenic mice compared to age matched C57Bl6 TRAMP $Tg^{+/-}$ x $MyD88^{-/-}$ mice.

Key Research Accomplishments

- We have shown that absence of MyD88 leads to increased formation of TRAMP prostate cancer tumors.
- We have shown that absence of MyD88 leads in increased tumor infiltration of CD11b+ cell types.

Reportable Outcomes

A lecture during the UCLA Jonsson Comprehensive Cancer Center Prostate SPORE Lecture Series, describing our preliminary work on inflammation in the tumor microenvironment.

Conclusions

Our initial crosses with TRAMP Tg mice and MyD88 deficient mice result in development of autochthonous prostate cancers opposite of what is observed in other *de novo* tumor models, suggesting the TLR signaling pathways have distinct properties in different tumors and demonstrates unique importance in prostate cancer that may influence disease progression and response to immune-based therapies.

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